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Towards the overall goal of making an accurate mathematical model of a neocortical pyramidal neuron, (1) the electrical properties of Na^+ and Ca^{2+} channel subtypes were measured in sufficient detail to construct a quantitative empirical model, and (2) antibodies were raised against the a_1 subunits of these channel subtypes in order to determine their spatial distribution and relative density using quantitative immunocytochemistry.

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FINAL TECHNICAL REPORT

ONR SPONSORED RESEARCH: *ROLE OF SPATIALLY DISTRIBUTED ION CHANNELS IN SINGLE NEURON COMPUTATION*

AWARDED TO PETER C. SCHWINDT, PH.D. (P.I.) AND WILLIAM A. CATTERALL, PH.D. FOR THE PERIOD MARCH 1, 1990 TO DECEMBER 15, 1992

Prepared By

Peter C. Schwindt, Ph.D.

INTRODUCTION

The overall purpose of this research was to elucidate the role of spatially distributed ion channels, specifically voltage-gated Na^+ and Ca^{2+} channels, in the transformation of synaptic input to spike output in neocortical pyramidal neurons. The immediate goals during this grant period were to determine (1) the electrical properties of these ion channels and (2) their spatial distribution and relative density along the initial segment, soma and dendrites. Ultimately, these quantitative measurements can be combined to create a mathematical model of the pyramidal neuron. This model can be analyzed to determine the functional consequences of the spatially distributed channels on neuronal excitability. Such a model is needed because complex spatio-temporal interaction between ion channels at different locations preclude functional analysis by direct recording of electrical activity.

The determination of ion channel properties was done in the laboratory of Dr. Schwindt. These studies consisted of whole-cell and single-channel recordings of ionic currents associated with channel activity. These studies focused on the persistent Na^+ current, whose mechanism was previously unknown, and the current flowing through L-, N- and P-type Ca^{2+} channels. The goal of these studies was to obtain sufficient quantitative data on the electrical properties of these channels to construct a quantitative empirical model suitable for use in the computer model of the whole-neuron referred to above.

The determination of ion channel location was done in the laboratory of Dr. Catterall. These studies consisted of the development of (1) specific antibodies directed against the α_1 subunits of specific Na^+ and Ca^{2+} channels subtypes and (2) a labeling procedure to visualize the antibodies the cell membrane and allow quantitative measurements of location and relative density of each channel subtype. The goal of these studies was to provide the spatial information needed for the whole-neuron model referred to above.

The results achieved during this grant period are summarized below in two sections, the first section describes the electrophysiological results, and the second describes the localization results. In this summary, I refer to published papers supported by this grant. These papers are numbered and listed alphabetically beginning on page 3. Reprints of those papers which have already been published are included with this report.

RESULTS

Electrophysiological Studies

Our original hypothesis was that the persistent sodium current, I_{NaP}, arose from a unique type of Na^+ channel, distinct from the one responsible for the transient Na^+ current and corresponding perhaps to the RI Na^+ channel subtype which is confined to the neuronal soma. As described in References 1-3, we found only one type of Na^+ channel on the soma of neocortical pyramidal neurons. This Na^+ channel displayed the usual transient gating mode, but two additional gating modes were apparent during long-

lasting depolarizations. Computations based on the single-channel data predicted that the late gating modes should give rise to a persistent whole-cell Na^+ current (3), and this was confirmed subsequently by whole-cell recordings from the same type of neurons (4,5). The two late gating modes appear to give rise to two kinetically-distinct components of I_{NaP} (5). It is unlikely that only the RI channel causes I_{NaP}. The late gating modes and the whole-cell I_{NaP} are seen both at early ages where only the RIII Na^+ channel type is present and also at later times when the RI channel type should also be present. Other researchers working on Na^+ channels from muscle and even cloned Na^+ channels have observed similar modal gating. Thus, it appears that any Na^+ channel subtype can give rise to an I_{NaP}, and we need no longer distinguish between Na^+ channel subtypes (e.g., RI, RII, etc.) in our single-neuron labeling studies. This will greatly expedite and simplify the gathering of the required data. Whole-cell recording has resulted in a description of the conductance underlying I_{NaP} that will be useful for modeling purposes (5).

Calcium Channels

The goal of this research was to construct an empirical model of the high-threshold voltage activated Ca^{2+} current (HVA current) of neocortical pyramidal neurons. These studies also have been completed (6,7). The HVA current is composed of pharmacologically-separable components, which probably correspond to activation of L, N and P channel types. Thus, an important question was whether these components differ in kinetics and voltage dependence. We have found that the HVA current of neocortical neurons may be treated as homogeneous with respect to these properties. That is, all components have the same voltage dependence and kinetics, irrespective of their sensitivity to pharmacological agents. This finding will greatly simplify the analysis of the role of these channels in neuronal computation once quantitative measures of channel density are available. Based on our measurements, we constructed a Hodgkin-Huxley-like model of the HVA current, and we found that this model could satisfactorily predict the HVA current evoked both by voltage clamp and a train of action potentials in dissociated neocortical neurons (7).

Ca^{2+}-activated Channels

Two other studies of the effects of Ca^{2+} influx and accumulation in neocortical pyramidal cells were performed during this period. In one of these studies (13), two distinct types of Ca^{2+}-activated K^+ currents in neocortical neurons were described in detail, and it was concluded that the dependence of one of these currents on Ca^{2+} was indirect, possibly through a Ca^{2+}- dependent enzyme intermediary. The other paper (12) showed that the response properties of the neurons is highly dependent on intracellular Ca^{2+} buffering in an unexpected way. In addition, two voltage-gated currents (including I_{NaP}) appear to be modulated indirectly by intracellular Ca^{2+} levels.

Other Work

At the invitation of our ONR Scientific Officer, Dr. Thomas McKenna, a book chapter was written describing the control of input-output properties of neocortical neurons by intrinsic membrane conductances (11).

Localization of Ion Channels

Calcium Channels

At the start of this project our sole available Ca^{2+} channel antibody was directed against the α_2 subunit of the L type Ca^{2+} channel in brain. During this grant period extensive work has been done on the identification and characterization of the various subtypes of Ca^{2+} channels. The work which resulted in publication thus far includes the determination of the primary structure of the rbB-I Ca^{2+} channel α_1 subunit (also known as class B Ca^{2+} channels). A polyclonal antiserum against this protein selectively

immunoprecipitates ^{125}Labeled ω-conotoxin-binding sites from rat forebrain. Expression of the rbB-I channel is restricted to the nervous system and cell lines that express the N-type Ca^{2+} channel (8). In a related study, a site directed anti-peptide antibody, CNB1, that recognizes the α_1 subunit of rat brain class B Ca^{2+} channels (rbB-I) immunoprecipitated 43% of the N-type Ca^{2+} channels labeled by ^{125}I-ω-conotoxin. In addition, the CNB1 antibody recognized proteins of 240 and 210 kd, suggesting the existence of two forms of this α_1 subunit. Immunocytochemical studies demonstrated that N-type channels recognized by CNB1 were localized predominantly in dendrites; both dendritic shafts and punctate synaptic structures were labeled. The cell bodies of some pyramidal cells in layers II, III, and V of dorsal cortex, Purkinje cells, and scattered cell bodies elsewhere in the brain were also labeled at low levels (14, 15).

In another study we have identified and localized the protein product of the two different class C and class D L-type Ca^{2+} channel α-1 subunits. Two antipeptide antibodies specific for the class C or class D α_1 subunits, known as CNC1 or CND1 respectively, were produced. Fully 75% of the neuronal L-type channels labeled by 3H-PN200-110 could be immunoprecipitated by CNC1, and 20% was immunoprecipitated by CND1. Immunoblotting revealed the existence of two sizes of the Class C L-type α_1 subunit and two sizes of the class D L-type α_1 subunit. Immunocytochemical studies using CNC1 and CND1 antibodies revealed that the α_1 subunit of both L-type Ca^{2+} channels is localized mainly on neuronal somata and proximal dendrites (10). Staining in distal dendrites was usually very faint but detectable. The relatively high concentration of L-type Ca^{2+} channels in cell bodies and proximal dendrites is in contrast to the predominant localization of N-type Ca^{2+} channels in distal dendrites.

Because of these exciting new developments we are able to greatly expand the scope of our investigation. Up to now we could only label the α_2 subunit of L channels. Not only can we more accurately localize subtypes of these L channels (by using α_1-directed antibodies), but we now will be able to visualize the spatial location of the three Ca^{2+} channel subtypes (L, N and P) that are known to underlie the HVA Ca^{2+} current in neurons (9).

Sodium Channels

Measurement of the spatial localization of Na^+ channels in single neurons has proceeded using confocal microscopy. In light of our physiological results (see above) suggesting that any type of Na^+ channel can give rise to a persistent Na^+ current, we can now utilize an antibody (AbSP20) that recognizes all types of Na^+ channels. This will expedite and simplify the gathering of the required data.

Published Material Resulting From ONR Funding:

1. Alzheimer, C., Schwindt, P.C. and Crill, W.E. Brain sodium channels occasionally fail to inactivate. Pfluegers Archiv, Suppl. A, 40: R27, 1992.

2. Alzheimer, C., Schwindt, P.C. and Crill, W.E. Two gating modes of Na channel openings in neurons from rat neocortex. Abstracts, Biophysical Society, 1992.

3. Alzheimer, C., Schwindt, P.C. and Crill, W.E. Modal gating of Na+ channels as a mechanism of persistent Na+ current in pyramidal neurons from rat and cat sensorimotor cortex. Journal of Neuroscience 13: 660-673, 1993.

4. Alzheimer, C., Schwindt, P.C. and Crill, W.E. Postnatal development of a persistent Na^+ current in pyramidal neurons from rat sensorimotor cortex. Journal of Neurophysiology 69: 290-292, 1993.

5. Alzheimer, C., Schwindt, P.C. and Crill, W.E. Composite nature of a persistent sodium current in rat neocortical neurones: relation to two modes of late sodium channel gating. (submitted 1993).

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7. Brown, A.M., Schwindt, P.C. and Crill, W.E. Voltage dependence and activation kinetics of pharmacologically defined components of the high threshold calcium current in rat neocortical neurons. (submitted, 1993).

8. Dubel, S.J., Starr, T.V.B., Hell, J., Ahlijanian, M.K., Enyeart, J.J., Catterall, W.A., and Snutch, T.P. Molecular cloning of the α-1 subunit of an ω-conotoxin-sensitive calcium channel. Proc. Natl. Acad. Sci. USA 89: 5058-5062, 1992.

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12. Schwindt, P.C., Spain, W.J. and Crill, W.E. Effects of intracellular calcium chelation on voltage-dependent and calcium-dependent currents in cat neocortical neurons. Neuroscience 47: 571-578, 1992.

13. Schwindt, P.C., Spain, W.J. and Crill, W.E. Calcium-dependent potassium currents in neurons from cat sensorimotor cortex. Journal of Neurophysiology 67: 216-221, 1992.

14. Westenbroek, R.E., Hell, J.W., Dubel, S.J., Snutch, T.P. and Catterall, W.A. Distribution of ω-conotoxin-sensitive calcium channels in the adult rat brain. Abstracts, Society for Neuroscience. 18: 971, 1992.

15. Westenbroek, R.E., Hell, J.W., Warner, C., Dubel, S.J., Snutch, T.P. and Catterall, W.A. Biochemical properties and subcellular distribution of an N-type calcium channel α-1 subunit. Neuron 9: 1099-1115, 1992.